

Observations on the Corneal Nipples in Mosquitoes (Diptera)

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RECEIVED FOR PUBLICATION MAY 4, 1971

Abstract: The ommatidia of *Aedes aegypti* are densely covered with corneal nipples which are rather regularly arrayed over the surface. Although there was no special distribution pattern the inter-ommatidial spaces of the male *A. aegypti* had nipple-like projections. It is possible that the nipples may be scratched off the cornea with no damage to the underlying structures.

The compound eye of the mosquito *Aedes aegypti* L. is composed of approximately 400 ommatidia (Figs. 1 and 2) with more in females than in males (Christophers, 1960). Each ommatidium is made up of an external dioptric apparatus and an underlying receptor layer of retinula cells (Brammer, 1970). The dioptric apparatus consists of an outer hardened cuticular cornea, a bi-convex lens, and associated pigment cells. Its function is the transmission of light to the rhabdomere—that portion of the cell in which the photosensitive visual pigment is located (Goldsmith, 1964; Langer, 1966; Langer & Thorell, 1966).

The initial structure which light strikes as it enters the eye is the cornea. The corneal surface of each ommatidium is covered with an array of protuberances, first described in a night moth by Bernhard & Miller (1962), who called them corneal nipples. Since that time corneal nipples have been seen in a wide variety of insects. Indeed, grouping of insects according to corneal types has recently been accomplished on the basis of examination of 361 different species from most insect orders (Bernhard, Gemne & Sällström, 1970). The present report deals with observations which have been made on the corneal nipples in various species of mosquitoes.

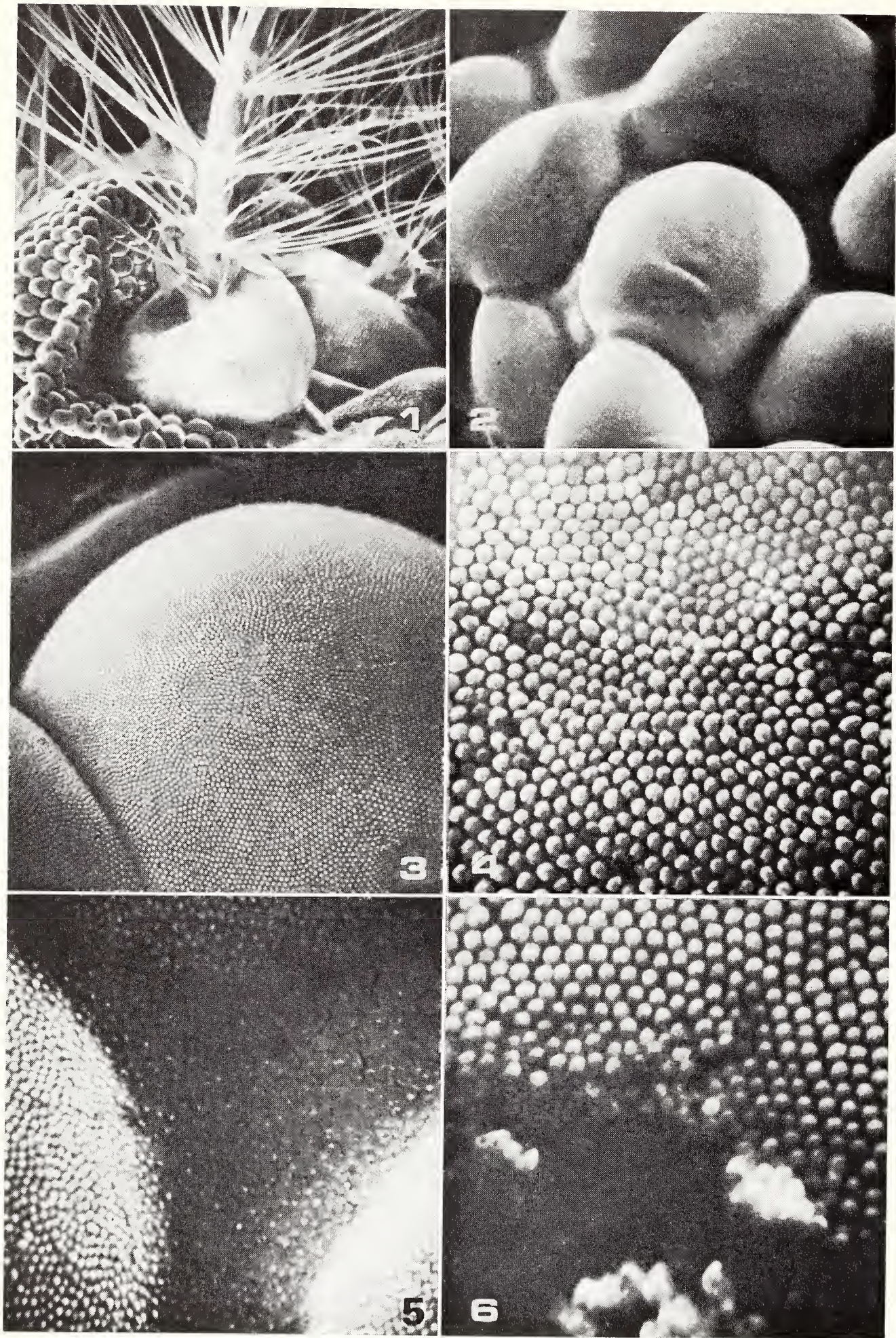
MATERIALS AND METHODS

Adult *Aedes aegypti* mosquitoes were obtained from eggs supplied by Dr. George Craig, Jr. of the University of Notre Dame. They were examined by both conventional transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). Males and females of 7 other species of mosquitoes obtained from field collections were also examined by SEM. These were *Aedes excrucians*, *A. vexans*; *Anopheles quadrimaculatus*, *A. sinensis*; *Theobaldia melanura*; *Culex pipiens* and *Masonia perturbans*.

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Compound eyes were prepared for TEM by fixation for one hour in cold 5 percent veronal buffered glutaraldehyde, followed by a thirty minute buffer wash and an additional one hour fixation in cold 1 percent buffered osmium tetroxide. They were left overnight (12 to 18 hours) in cold buffer wash, and the following morning were again placed in 1 percent osmium tetroxide for one hour. They were then dehydrated in a graded series of cold acetone and embedded in an Epon-Araldite mixture (Mollenhauer, 1964). Sections were cut on glass knives and stained with uranyl acetate (saturated in the 70 percent acetone dehydration step) and lead hydroxide (Millonig, 1961). The sections were examined either with a Hitachi HU 11A or a Phillips 200 electron microscope.

The compound eyes were prepared for SEM by using aluminum paint to cement the dried specimens to stubs in such a position that both eyes could be viewed by rotation of the stub. All surfaces were coated in a vacuum chamber with approximately 200 Å of gold. Specimens were examined with a Cambridge Steroscan Mark II SEM.

RESULTS AND DISCUSSION

Scanning electron microscopy reveals the ommatidia are densely covered with corneal nipples (Figs. 3 and 4) which are rather regularly arrayed over the surface of each ommatidium. Measurements made from nipples which have been cut longitudinally and examined by TEM show that they are about 200 μ in height and 150 μ across at their base (Fig. 7). These observations agree with those made by Bernhard et al. (1970), who found similar-sized nipples on the cornea of several species of mosquitoes.

There appears to be no special pattern to the nipples with respect to either the sex of the mosquito or to the different species studies. However, it was noted that in the inter-ommatidial spaces of male *Aedes aegypti* many nipple-like projections are found (Fig. 5). No such projections have been seen in the males of other species examined, nor in any of the females. It is difficult to think of a functional role in vision which these nipple-like projections might serve, for secondary pigment cells are the only structures found below the cuticle in the inter-ommatidial spaces. The function of these secondary pigment cells is the screening of oblique light rays from the photoreceptive portion of the ommatidium (Höglund, 1965).

The appearance of the inter-ommatidial projections in the males, but not in the females, may be related to developmental differences between the two sexes. In the developing eye of both sexes the primary pigment cells are first found lying distal to the cone cells, and are involved in the formation of the

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FIGS. 1-6. *Aedes aegypti* ♂. FIG. 1. Ommatidia and antennae 100×. FIG. 2. Ommatidia 2,000×. FIG. 3. Ommatidia showing corneal nipples 5,000×. FIG. 4. Corneal nipples 10,000×. FIG. 5. Inter-ommatidial projections 6,000×. FIG. 6. Corneal nipples scratched from surface of ommatidia 10,000×.

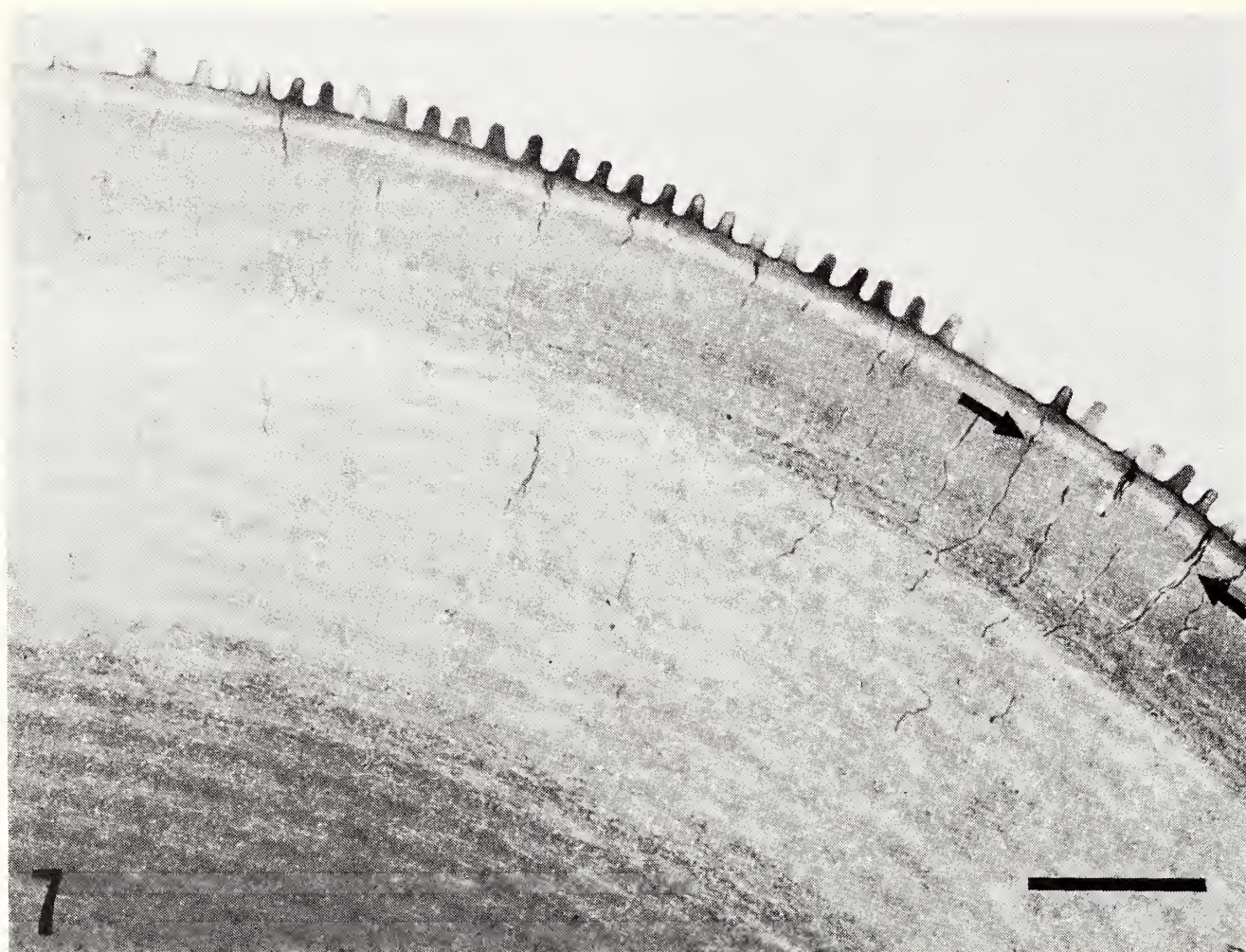


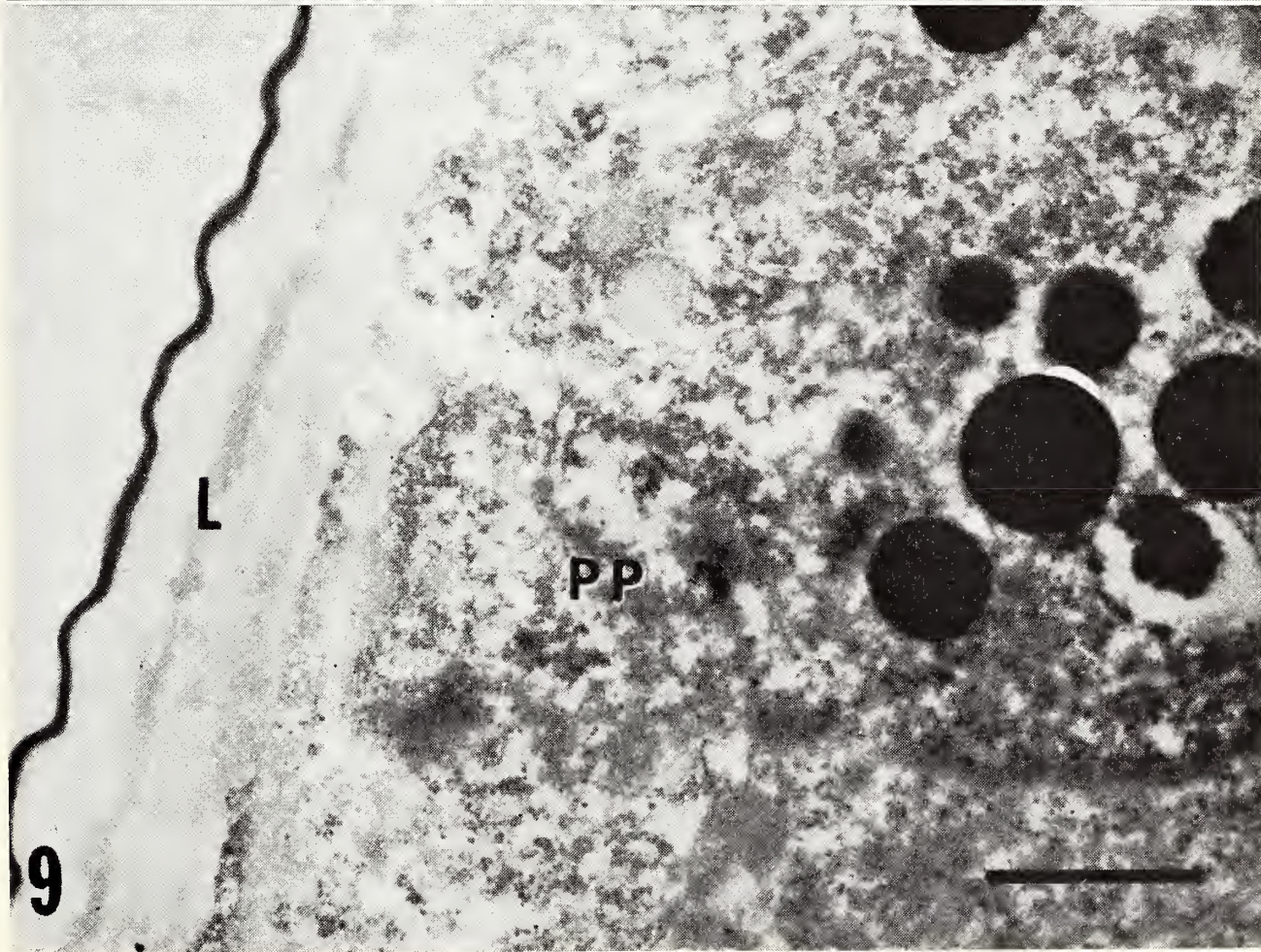
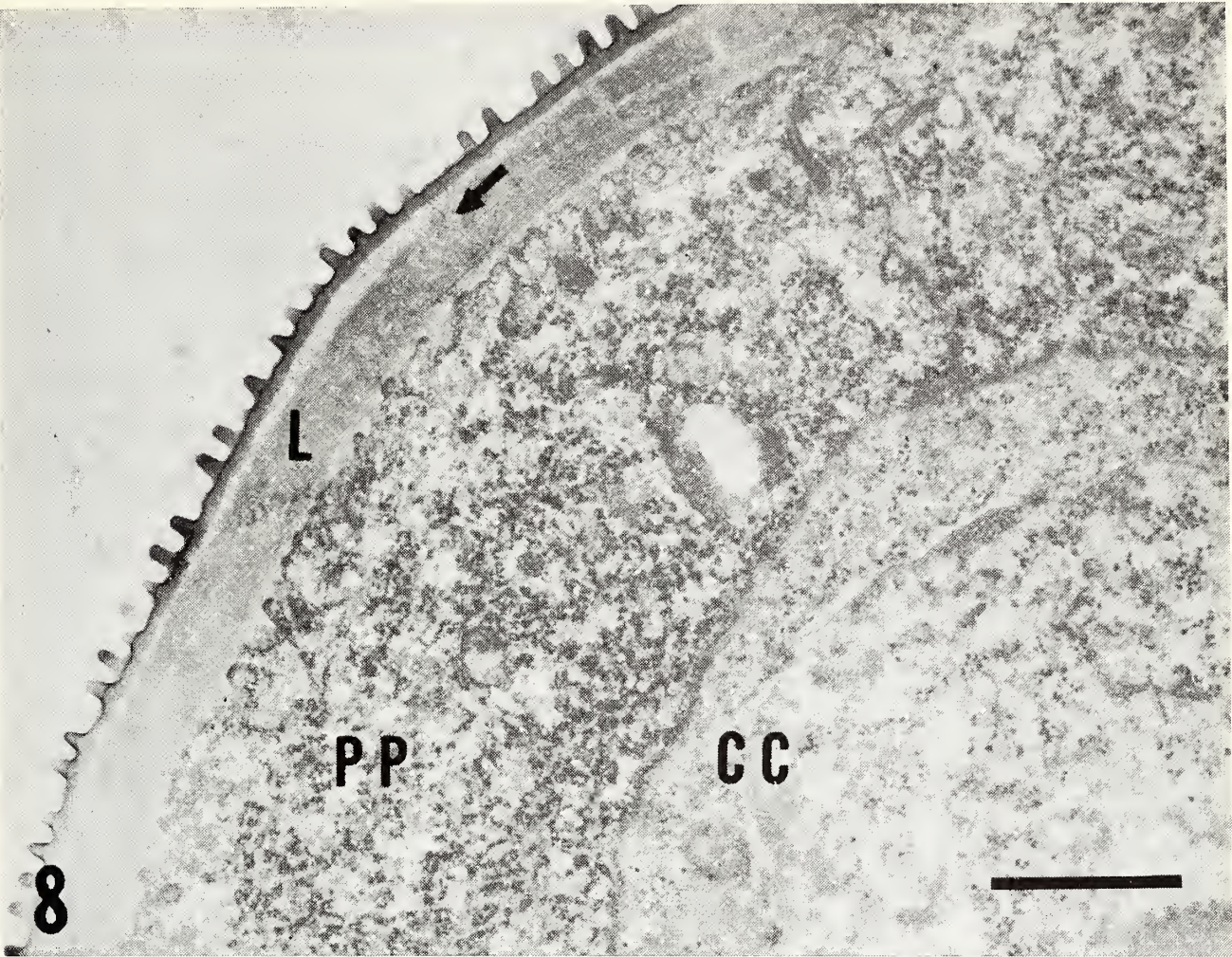
FIG. 7. The cornea and a portion of the lens in an adult *Aedes aegypti*. The plane of the section is at right angles to the surface of the eye. Epicuticular filaments are indicated by arrows. 10,000 \times .

cornea and the lens. As development proceeds, however, the primary pigment cells move peripherally, become pigmented, and eventually completely surround the cone (Goldsmith, 1964). In *Aedes aegypti* adults, the males are smaller in size than the females, and this is also true of their ommatidia. The reduction in ommatidial size, however, is not fully proportional to the smaller size of the female (Christophers, 1960). It is possible, then, that in the developing eye

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FIG. 8. The developing cornea and lens in the mid-pupal stage of *Aedes aegypti*. The arrow indicates an epicuticular filament which passes completely through the lens material (L) that has already been deposited. The underlying epidermal cells (PP), which will become the primary pigment cells of the adult ommatidium, have their surface adjacent to the lens material thrown into numerous folds. A cone cell (CC) with its large nucleus is seen at lower right. The solid line represents one micron. 10,000 \times .

FIG. 9. The developing eye in a very early pupal stage of *Aedes aegypti*. Lens material (L) is being deposited in small amounts by the future primary pigment cells (PP), but no corneal nipples can be seen at this time. The solid line represents one micron. 10,000 \times .



of male pupae the primary pigment cells cause the formation of nipples in an area of cuticle larger in size than actually needed to form the cornea over each ommatidium in the adult, while such is not the case in the females. Although entirely speculative, this could explain the appearance of nipple-like projections in the inter-ommatidial spaces of males, but not females in *A. aegypti*.

In eyes which have been sectioned longitudinally the material within each nipple appears much the same as that found in the cortical region of the lens (Fig. 7). Also seen in this plane are filaments which are oriented at right angles to the surface of the eye. These filaments are confined to the superficial region of the adult lens. They are less prominent in the developing lens of the pupae, but nevertheless often extend through the entire layer of the lens material which has been deposited (Fig. 8). The filaments do not penetrate the body of the nipples, but rather make contact with the corneal surface between the nipples (Figs. 7 and 8). Similar filaments have been described in other regions of insect cuticle. Locke (1966) called such structures wax canal filaments, but Filshie (1970) suggests they should be called epicuticular filaments, since they are also found in certain insects whose cuticle lack the waxy layer.

The surface of the primary pigment cells which lie just beneath the developing lens is thrown into a series of folds, or microvilli. These microvilli are easily seen in mid-stage pupae before much lens material has been deposited, but yet after the nipples are well formed (Fig. 8). Although sometimes present, they are much less obvious in very early pupae, and no nipples can be seen at this time either (Fig. 9). Microvilli have previously been associated with corneal nipple formation in other insects (Gemne, 1966). Similar microvilli are also present between the lens and the underlying cone cells in the adult ommatidium (Brammer, 1968).

It appears possible that the nipples can be scatched off the cornea with no apparent damage to the underlying structures, for vacant areas are sometimes seen on the ommatidial surfaces with the SEM (Fig. 6). This may also explain why some nipples appear to be absent in TEM micrographs (Figs. 7 and 8).

The function of the corneal nipples in mosquitoes is still speculative. Microwave experiments on dielectric models of the lens (Bernhard, Miller and Møller 1963, 1965), combined with spectrophotometric measurements made from pieces of cornea (Miller, Møller and Bernhard, 1966), have shown that the nipples act as an impedance transformer which enhances transmission of visible light into the eye. Although the increase in transmitted light is relatively small (about 5 percent of the incident light), it may be of significance in those insects such as mosquitoes which are active at night.

It may also be possible to apply the principles of fiber optics to the corneal nipples. Kapany (1960) states that when one end of a fiber bundle is fitted to a curved surface, and the other end to a flat surface, the image delivered at the

focal plane behind the curved surface is not only undistorted, but also is sharper and brighter. If the corneal nipples which are regularly scattered over the curved surface of the ommatidium behave as fiber optics, and if the transmission characteristics of the underlying lens is appropriate, a sharper, brighter image could be delivered to the rhabdom than would have been possible in the absence of the nipples. The studies of Bernhard et al. (1963, 1965, 1966), while showing the role of the nipples with respect to light transmission, would not necessarily have detected any contributions they might make toward increased resolution.

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